

Research on the culturing of the brine shrimp Artemia salina L. at the State University of Ghent (Belgium)

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Abstract

In the framework of research on mariculture at the "Institute for Marine Scientific Research" (I.Z.W.O.), the "Laboratory for Biological Research in Environmental Pollution" at the State University of Ghent, is focusing on culturing problems of the brine shrimp *Artemia salina*.

The following research topics are discussed:

1. The mechanisms leading to the hatching of *Artemia* cysts are reviewed and complemented with new data on the sensitivity to light and temperature, and on the reversibility of the development within the cyst. The possible influences of the collecting, sorting, and packaging processes on the quality of the cysts are discussed.
2. Since in the literature much confusion exists concerning the mechanisms responsible for the type of reproduction (oviparity or ovoviviparity), the influence of the parameters light, oxygen, and salinity were studied.

From the results it is already clear that:

- neither the light intensity, nor the photoperiod influence the type of reproduction;
- the total number of offspring is definitely correlated with the light intensity, *i.e.* a higher number of offspring is produced in darkness as compared to in light;
- low concentrations of dissolved oxygen induce the formation of haemoglobin with a subsequent shift of the mode of reproduction from ovoviviparity to oviparity.
- following the law of Henry concerning the solubility of gases, the oxygen concentration in saline waters is inversely proportional to the salinity. This explains the natural production of cysts in hypersaline salt ponds. The oxygen level may not, however, drop below a certain level, that is lethal for the adult *Artemia*. This means, that the mass culture of *Artemia* for the production of cysts, will require rather constant or controlled environmental conditions, especially in outdoor ponds.

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3. Preliminary studies on *Artemia* strains from different sites all over the world, reveal important differences from one race to another in hatching rate, length of the newborn larvae, growth rate, sensitivity, etc. In order to be able to carry out a detailed comparative analysis of the biological and biochemical characteristics of all 40 different *Artemia* strains, a method using a factorial design and a response-surface analysis was developed allowing the determination of the optimum temperature-salinity combination.

The preliminary data presented on strains from the U.S.A., Bulgaria, and China reveal that considerable differences exist between these races with regard to the survival of nauplii in function of the temperature and the salinity.

Introduction

For several years the larvae of the brine shrimp, *Artemia salina* L. can rightly be called the most common feeding tool in mariculture work. Most of the applied techniques, however, are quite primitive and based on trial and error. Specific fundamental studies on *Artemia* as a baseline for maricultural applications are scarce, and most people still do not seem to realize that there is an urgent need for research on this live food which is of crucial importance for the future development of fish and crustacean aquaculture throughout the world.

When in 1969 we started to tackle some fundamental biological aspects of brine shrimp culturing, we often had to cope with the remark that "everything was already known on *Artemia*". Within a few years, however, a number of important phenomena in the hatching of the cysts were discovered, and improved techniques for hatching the larvae, for separating the hatching debris from the organisms, and for the high density culturing of the larvae could be developed (Sorgeloos and Persoone, 1975).

Considering the increasing worldwide use of brine shrimps, the fundamental as well as applied research on *Artemia* in our laboratory, has been much expanded. Presently an entire research-team is focusing on various aspects of *Artemia* culturing which will be briefly discussed.

Hatching of the nauplii : some facts about the cyst-metabolism

In previous papers (Sorgeloos, 1973a; Sorgeloos and Persoone, 1975), we reported on the triggering effect of light on the metabolism of *Artemia* cysts. By illuminating the cysts shortly after hydration, the hatching efficiency increased by 30-40 %, as compared to the hatching efficiency of cysts kept continuously in darkness.

Since in many hatching devices described in literature, the cysts are immersed in non-transparent containers (Shelbourne *et al.*, 1963; Riley, 1966; Shelbourne, in Costlow 1969; Jones, 1972; Nash, 1973), we have been wondering, when considering the literature on the cyst's metabolism, if it would not be possible to treat the cysts in some way, prior to packaging, to ensure a maximal hatching efficiency at a later immersion in complete darkness:

— From Dutrieu (1960), Dutrieu and Cresta-Blanchine (1966) and Clegg (1967) it appears, that as long as the breaking of the shell has not happened, the cyst can be hydrated and dehydrated reversibly, or repeatedly hydrated in aerobic conditions followed by anaerobic ones.

—Morris (1971) showed that the effects of repeated hydrations on the embryonic development are cumulative: the time until hatching is shortened when several hydrations are applied.

—Our light studies revealed, that the cysts only need a definite irradiance stimulus shortly after full hydration (e.g. a minimal irradiance time at 2,000 lux of respectively 5 and 10 min for cysts from San Francisco Bay, California, U.S.A. and from Great Salt Lake, Utah, U.S.A.).

Considering these three facts, we assumed that the effect of light after the initial hydration, is probably stored for later immersions, which can then of course be carried out in conditions of continuous darkness.

The first experimental results concerning this hypothesis were hopeful: San Francisco Bay cysts hydrated for 6 hr under continuous illumination and subsequently dried for 24 hr, gave a maximal hatching efficiency of 90 % when immersed immediately afterwards in continuous darkness.

When the cysts treated as described above were, however, kept dry or were hydrated in anaerobic conditions for longer periods (weeks), the hatching efficiency in darkness decreased significantly with time of storage.

We furthermore observed, that the time till hatching was significantly longer for those cysts that had been dried for weeks compared to those dried for only 24 hr after an initial hydration.

As this latter observation is in contradiction with the theory of Morris (1971), on the cumulative effect of the *Artemia* cyst's metabolism, we studied the reversibility aspect of the cyst's development in greater detail.

Although this research is still in progress, we can already mention the following interesting findings:

1. There are several alternatives to the two methods known from literature, to reversibly interrupt the cyst's metabolism [drying of the active cysts (Dutrieu, 1960; Clegg, 1967) or transfer of the cysts to anaerobic seawater (Dutrieu and Cresta-Blanchine, 1966)]. Among those alternatives two techniques are very practical: dehydration of the cysts in a brine solution of 280 g NaCl/l or transfer into a 3 % sodium sulphite solution (made up with distilled water).
2. A third mechanism to reversibly interrupt the cyst's metabolism was discovered: namely the transfer of the active cyst to seawater at 40 °C.
3. The effect of repeated metabolic activities of cysts hydrated in optimal physical-chemical conditions (salinity, temperature, dissolved oxygen, and light) is only cumulative for short phases of interruption (i.e. hours in the case of an interruption due to an increase of the temperature, or weeks after dehydration or anaerobic conditioning).

There appears to be a significant difference in reactivity of the cysts, depending on the way in which the previous interruption of the cyst's metabolism was achieved.

Let us consider a few examples:

- a. Cysts which are dehydrated for 24 hr after an initial immersion period of 8 hr (in optimum conditions) reach the breaking phase after a second metabolic cycle of only 5 hr. In this case, there is a definite cumulative effect since the normal time from immersion until breaking is approximately 13 hr.

- b. When the metabolism is interrupted for 15 weeks after an initial hydration as described above, the second cycle of active metabolism takes about 10 hr. The total hydration time till the breaking in this case, is 18 hr *versus* 13 hr for non-treated cysts.
 - c. Interruption of the cyst's metabolism by transfer of the cysts to anaerobic conditions instead of dehydration, gives exactly the same results as mentioned in a and b.
 - d. The effect of the interruption is much more drastic in the case of metabolism interruption by a temperature increase to 40 °C, than by dehydration or anaerobic conditions. Immersion of cysts in seawater of 28 °C for 8 hr → transfer in 40 °C seawater → interruption period of 24 hr → transfer in 28 °C seawater → breaking stage after 12 hr. The total hydration time in optimal conditions appears to be 20 hr, thus meaning a delay of 7 hr.
 - e. In experiments analogous to d but with an interruption period of 3 days, the breaking only occurs 30 hr after the transfer from 40 to 28 °C. The total hydration time under these conditions is 38 hr.
4. A considerable delay in the time till hatching can also be induced by hydrating the cysts at 40 °C for 3 days.
- The latter two observations (3e and 4) indicate that a temperature increase up to 40 °C does not only interrupt the cyst's activity but also in some way retrogrades the biochemical processes occurring in the embryo.
5. Although the hatching efficiencies were not calculated, no obvious decrease in total percentage of hatching seemed to occur in any of the previous experiments. When repeated temperature-cycles (8 hr at 28 °C followed by 24 hr at 40 °C) were applied, the percent hatching dropped, however, markedly.
6. Last but not least, we observed that the average length of the first instar nauplii hatched from cysts that had been submitted to repeated temperature cycles (*cf.* 5), were significantly smaller than those hatched from non-treated cysts.
- By further increase in the number of temperature cycles, we observed that gradually the cysts did not reach the hatching stage, respectively the breaking stage. In the former case the nauplii seemed to be unable to disrupt the hatching membrane and died.

These observations seem to reveal, that the total energy content of the cysts decreases by repeated applications of temperature cycles.

Although the results obtained are still preliminary and need to be complemented by biochemical data, we can already try to extrapolate eventual practical aquacultural applications. For example there seems to be sufficient evidence provided to assume that metabolic activity occurs more or less frequently in cysts, which accumulate along the shores of the salt lakes. They can *e.g.* be hydrated more or less regularly by rainfall, be submitted to cyclic temperature changes, *etc.*

Further experimentation should prove if the important differences in hatchability from one batch of cysts to another, can be explained by the formulated hypothesis. Even when the hatching efficiencies of the batches collected in the wild are optimal, their biochemical composition (and thus their nutritional value) can vary considerably.

As a result, one should seriously consider either to harvest the cysts more frequently, or to prevent them from accumulating on the shores, where they are much more exposed to the mechanisms mentioned above, that induce the metabolic activity, than when they remain in the water where they will not hatch until the salinity drops to below 70‰.

Reproduction in *Artemia* : oviparity versus ovoviviparity

Since aquaculture as well as aquarium keeping as a hobby expand almost exponentially, it is expected that the supply of *Artemia* cysts, which presently cannot meet the demands, could become more and more a bottle-neck in the future.

Presently, the principal source of these cysts is the harvest of mainly unmanaged wild populations which are subject to strong environmental perturbations and only procure high productions at unpredictable moments, when the conditions are accidentally favorable (Helfrich, 1973).

During recent years, several project proposals to mass produce *Artemia* cysts in tropical and subtropical saline lakes, have been introduced.

It is clear, however, that extensive biological research is needed, starting with unraveling the regulatory mechanism of oviparity and ovoviviparity, before any of these projects has a serious change to succeed. Indeed, to date, still much confusion exists concerning the mechanisms of reproduction in *Artemia*.

Several parameters have been set forward as being determinative for the mode of reproduction: salinity was mentioned by Abonyi (1915) and Barigozzi (1939); Dutrieu (1959, 1960), Lochhead (1961), and Baker (1966) correlated oviparity with the formation of haemoglobin; Grosch (1962) reported on the seasonal periodicity and Ballardin and Metalli (1963) thought that the mode of reproduction was influenced by bad environmental conditions. D'Agostino (1965) and D'Agostino and Provasoli (1968) stated that oviparity was induced by the food quality and/or quantity. Von Hentig (1971) found no correlation with the external conditions. Last but not least, Metalli and Ballardin (1972) reported that "... the physiological conditions of the female which are responsible for the choice of viviparity *versus* oviparity for each brood of eggs, and their relationship with environmental conditions seem to be very complex and they are also poorly understood. However, the overall frequency of oviparity is strongly strain-dependent, and this simple observation points to some basic mechanisms which are under genetic control".

In the light of the existing confusion, and considering the urgent need to arrive at a controlled cyst production, we have been studying the influence of the following parameters on the mode of reproduction: light intensity and photoperiod, dissolved oxygen concentration, and salinity.

Influence of continuous light, continuous darkness, and two different photoperiods on the mode of reproduction

From the literature it is known that in the cladoceran genera *Chydorus* (Shan and Frey, 1968) and *Daphnia* (Stross and Hill, 1965, 1968; Parker, 1966), and in several represen-

tatives of the order of Insects (Lees, 1959 ; Clever, 1962 ; Shilova and Zelentsov, 1972), diapause or cyst production is induced by a specific photoperiod. For this reason, we decided to examine the effect of the factor light on the mode of reproduction in *Artemia*.

1. Materials and methods

Great Salt Lake (Utah, U.S.A.) *Artemia* nauplii were cultured to the adult stage using a technique previously described (Sorgeloos, 1973b ; Sorgeloos and Persoone, 1975). The experiments were carried out with adults which had already given several offspring. Bowen (1962) and Von Hentig (1971) indeed reported that the first offspring is smaller than the subsequent ones which do not significantly differ from each other.

Four series of eight beakers each, containing 250 ml artificial seawater (formula of Dietrich and Kalle, 1963) were kept at 28 °C under the following regimes of respectively light or darkness : continuous light, continuous darkness, and the two photoperiods 16:08 and 08:16. The light intensity at the water surface was approximately 2,000 lux.

Each beaker contained two couples of adult *Artemia* which were fed twice daily with 1 ml of a suspension of *Scenedesmus* powder (1 g/l) ; details on the preparation of the food can be found in a previous paper (Sorgeloos and Persoone, 1975). The beakers were aerated twice an hour for 10 sec by air-bubbling, which at the same time resuspended the food particles. Every 5 days, the medium was renewed and the beakers controlled for the presence of offspring. The nauplii were fixed with a lugol solution and counted under a dissection microscope. White-grey colored "Subitaneier", which hatch shortly after their deposition, were occasionally found and counted as ovoviviparous offspring (cf. D'Agostino, 1965).

The entire experiment lasted for 1 month, the data on reproduction were only calculated for the last 3 weeks, the first 10 days being considered as an adaption period.

2. Results and discussion

In Table I, the number of larvae and cysts detected at each observation, the total reproduction (larvae + cysts) for each parallel, the number of produced offspring, and the standard deviation from the mean with the corresponding coefficient of variation for each series, are given.

From the results it is clear, that the photoperiod has no obvious influence on the mode of reproduction : in all the series ovoviviparity was dominant, only 8 % of the total offspring was deposited as cysts.

Since a simultaneous ovovivi- and oviparous reproduction does not occur in the same animal (Bowen, 1962 ; Nimura, 1967), it is clear that in those parallels where cysts were deposited, only one female was or became oviparous and kept that mode of reproduction (e.g. series 08:16, parallel 4).

The comparison of the number of offspring among the different conditions of illumination, reveals that at the $P < 0.1$ level, significantly more offspring was produced in the series in continuous darkness and at a light-dark regime of 08:16, than in the series with continuous light and light-dark regime 16:08. These findings have been fur-

TABLE I
The influence of continuous light, continuous darkness, and 08:16 and 16:08 photoperiods on the reproductive performance of *Artemia*. (L = larvae; C = cysts)

Conditions of illumination	Time (days)												Total number of offspring	Mean number \pm SD	Coefficient of variation (%)						
	2			4			6			8						10			12		
	L	C	L	L	C	L	L	C	L	L	C	L				L	C	L	L	C	
2,000 lux	192			262								414					868				
	280			395								396					1,071				
	164			232				68				94		178			718				
	85			97	194			251				10	91	185	191		1,004		16		
	34			27	241			357						384			1,043				
200 lux	63			174	221			205				30	181	165			1,039		15		
	164			68	229			94						178			733				
	209			368								434					1,017				
	65			178	132			170				304					849				
Continuous darkness	470			427				158				108		83			1,246				
	353			286								470					1,109				
	180	99		264			160					255	145				1,103		6		
	350			314				175				380					1,219				
	225			209				195				163		152			1,101				

ther studied in another experiment which is described later in this paper (see : "The influence of the light intensity on the reproductive capacity").

From the assumption, that one reproductive cycle occurs every 4-5 days, in the given set of environmental conditions, (cf. Von Hentig, 1971), we calculated the mean reproduction to be 181 young per brood, a figure which approximates the best data reported in literature (Ballardin and Metalli, 1963 ; Nimura, 1967 ; Von Hentig, 1971 ; Barigozzi, 1974).

The high figures for the coefficient of variation can, in our opinion, be explained by the non-synchronous reproduction of the animals: at the moment of the final observation at the end of the one month experiment, some females might indeed just have released an offspring, while others would have done it only one or a few days later.

An interesting observation is that light does not play an essential role in the initiation of precopulation (riding position) ; male and female indeed find each other even in total darkness, a fact which can possibly be attributed to chemoreception.

The influence of the light intensity on the reproductive capacity

"... the scarce papers on reproduction are dealing much more with reproductive mechanisms than with reproductive capacities" (Segal, 1970).

Buikema (1968, 1973) reported that in *Daphnia*, the light intensity significantly affects both the number of young per brood and the total offspring. Since in these experiments the animals were fed with live algae, which also react to the light conditions during the course of the experiment (cf. Spoehr and Milner, 1949 ; Taub, 1971), it is not excluded, that the differences in reproduction noted by this author, resulted at least partly, from the effect of light on the metabolism (and consequently from the nutritional value) of the algae, a hypothesis which was already formulated in 1956 by Green (1956).

1. Materials and methods

The experimental conditions were analogous to those described for the previous experiment, a few details excepted.

In order to obtain a more synchronous reproduction, the experiments were this time set up with adults which were developing their first offspring. The medium was renewed every 2nd day, and the produced number of offspring counted, and removed. The adaptation period lasted for 2 weeks and the experimental one for the subsequent 12 days. Three series, were set up each in five parallels: continuous darkness, and continuous illumination by a fluorescent light tube at 200 and 2,000 lux respectively. Due to technical difficulties, the results of one parallel in the 200 lux series cannot be taken into consideration.

2. Results and discussion

From the data in Table II, it is clear that:

- Ovoviviparity is the dominant mode of reproduction ; under the experimental conditions utilized only 6 % of the offspring was deposited as cysts.
- The reproductive capacity is higher in continuous darkness than under light con-

ditions. The difference between the dark series and the pooled results of the two illuminated series, is statistically significant at the $P < 0.05$ level. The difference between the two light series is, however, not significant.

- Calculated in the same way as in the previous experiment, the mean number of young per brood averaged 168, which is lower than in the first experiment but still high, when compared to the literature data on reproductive capacities.
- As the coefficient of variation is 16 % for both the series in light and only 6 % for the series in darkness, reproduction seems to be better in darkness.

The higher number of offspring in darkness can, in our opinion, be correlated with the differences in behaviour of the animals in darkness as compared to that in light. In the latter conditions, adult *Artemia* are indeed swimming very actively and show a strong gregarious behaviour. In darkness, on the contrary, they are less active and more evenly distributed throughout the water column.

The strong phototaxis may be the critical factor: indeed as soon as *Artemia* perceives a light stimulus, its activity increases. The couples apparently spend more energy for locomotion and consequently there is less energy left for reproduction. Above a certain threshold, and below a critical maximum, the activity remains constant. As the total number of offspring is equal for the 200 and the 2,000 lux series, both these intensities probably lie within the critical range.

We came to analogous conclusions in a previous study dealing with the influence of the light intensity on the growth rate of brine shrimp larvae (Sorgeloos, 1972).

The influence of dissolved oxygen and salinity on the mode of reproduction of Artemia

The work of Dutrieu (1960) is in our opinion the best study ever done on the reproduction of *Artemia*. Its conclusions can be summarized as follows: at low oxygen concentrations and favorable food conditions, blood haemoglobin is synthesised. Haematin, a derivate of haemoglobin is then excreted by the shell glands and incorporated in the egg shells of the diapause-embryos formed.

The experiments of Dutrieu (1960) were, however, not performed in strictly controlled or known conditions: the water in the aquaria was not aerated, nor renewed; the oxygen concentration was fluctuating irregularly; the adults were fed live algae which were subjected to and affected by the varying physico-chemical conditions, etc.

In order to test out the validity of Dutrieu's (1960) findings, under more controlled conditions, we constructed a culturing chamber in which the oxygen concentration of the medium could be precisely regulated. With this apparatus, the influence of the dissolved oxygen concentration on the mode of reproduction has been followed; the *Artemia*'s were fed on an inert diet to avoid some of the interferences mentioned above.

1. Materials and methods

The apparatus (schematic diagram in Fig. 1) consisted of a breeding vessel, a buffering cylinder, a peristaltic pump and a gas-mixing manometer, kept in a temperature conditioned room at 25 °C.

The 2 l buffering vessel and the 250 ml breeding vessel were filled with artificial seawater (formula of Dietrich and Kalle, 1963). The water was kept in continuous cir-

cultation by the peristaltic pump and flowed from the buffering vessel through the breeding chamber back into the first cylinder. A plankton gauze prevented the animals from swimming from the breeding vessel into the other compartments.

By bubbling a well defined mixture of air and nitrogen in the buffering vessel, the oxygen level of the medium could be kept constant within 0.05 ppm limits. Due to the small number of test-organisms and the continuous flow of the water, the oxygen concentration did not decrease significantly during its passage through the breeding vessel.

For each experiment, two couples of adult *Artemia* (Great Salt Lake strain) were brought in the breeding vessel. The animals were fed twice a day with dried

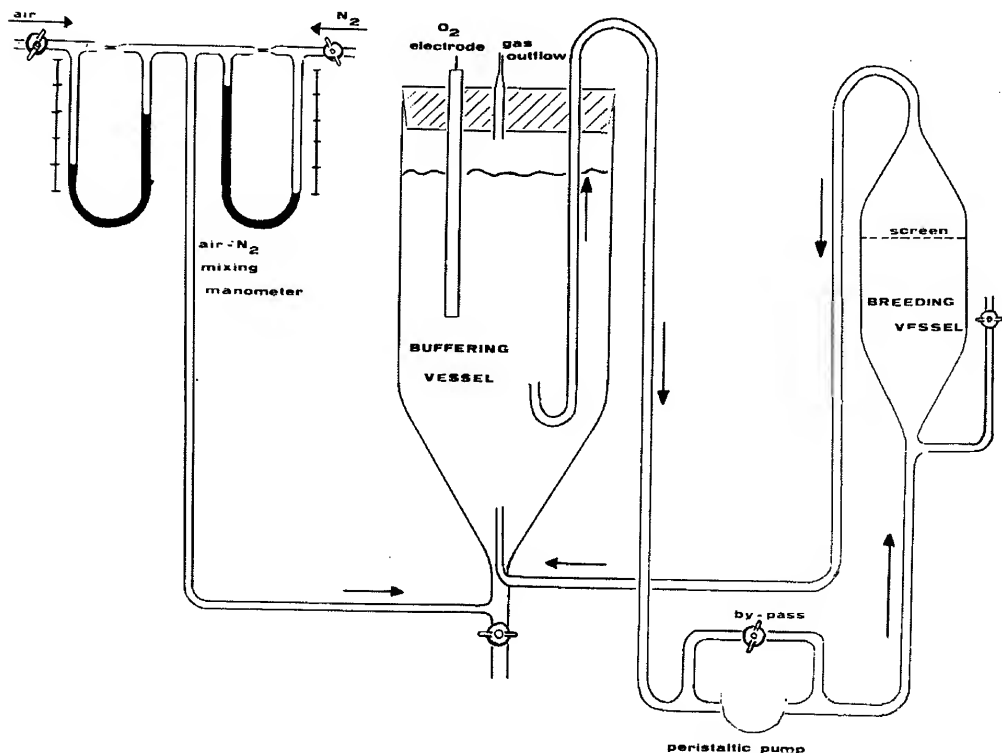


FIG. 1. Experimental setup for culturing couples of adult *Artemia* in controlled concentrations of dissolved oxygen.

Scenedesmus powder, and the content of the buffering cylinder was renewed every second day.

The mode of reproduction of the brine shrimp was followed during a period of 2 weeks at oxygen concentrations of respectively 8, 6, 4 and 2 ppm. As the stocks from which the test animals were taken had been cultured in seawater that was almost saturated with oxygen, it was necessary to progressively adapt the adults to the low oxygen levels. An adaptation period of 3 days for the 6 and 4 ppm experiment, and of 1 week for the 2 ppm test were taken.

2. Results and discussion

All females in the series at 8, 6 and 4 ppm dissolved oxygen produced free-swimming nauplii.

In the experiment at 2 ppm O_2 , however, the first reproduction was ovoviviparous whereas the three subsequent broods consisted of cysts.

Furthermore in the latter series, the adults were colored dark-red, which, according to Gilchrist (1954) and Dutrieu (1960) is due to a high concentration of haemoglobin in the blood.

Although these preliminary experiments should be replicated with larger numbers of animals, with adults reared in various dissolved oxygen concentrations, with adults from different geographical races, *etc.*, our present data corroborate the results of Dutrieu (1960) who also found that below a critical oxygen concentration, the reproduction mode switches from ovoviviparity to oviparity.

From our results the critical concentration appears to be situated between 2 and 4 ppm O_2 . Gilchrist (1954) detected a significant increase in the haemoglobin concentration when the dissolved oxygen concentration dropped below 4 ppm. Dutrieu (1960) collected cysts in aquaria with oxygen concentrations ranging from 0.5-3.6 mg/l. Katsutani (1965, personal communication) found the oviparous reproduction to be dominant in the salt lakes near Okayama City in those periods of the year, when the lowest dissolved oxygen concentrations were observed.

By extrapolation of the information available from the literature and from our own results, a valid theory on the natural regulation of the reproduction in *Artemia* can presently be formulated:

During certain periods of the year, the salinity in the salt lakes where *Artemia* populations thrive, is relatively low and the dissolved oxygen concentration high; the concentration of haemoglobin in the blood of these *Artemia* is then minimal, and ovoviviparity is the dominating mode of reproduction.

As the oxygen concentration in the water is not limiting, the nauplii (which do not have respiratory pigments) are able to survive under the prevailing conditions and to grow out to the adult stage.

At other moments of the year, however, the salinity of the salt lakes increases drastically (*e.g.* by evaporation). Following the physical law of Henry on the partial pressure of gases in water, the dissolved oxygen concentration decreases with increasing salinity. As the physiological availability of oxygen becomes restricted, the *Artemia* population starts to produce haemoglobin. At a certain threshold, the females shift from ovoviviparity to oviparity and deposit cysts which enter into diapause.

The oviparous reproduction (with formation of very resistant cysts) offers many important ecological advantages:

- Conservation of the species which otherwise would become extinct due to the extreme environmental conditions, and the lack of haemoglobin in the nauplii's blood (*cf.* experiments of Gilchrist, 1954).
- Possibilities of transfer of the cysts by winds or other carriers (birds) to other suitable biotopes (biogeographical distribution).
- Extension in time of the conservation of the gene pool by dormancy-periods that can last for many years.

In contradiction with the proposed theory, several authors have more or less exceptionally observed cysts production under conditions of high dissolved oxygen levels (*cf.* our results for the two previous experiments). Since Baker (1966) reported that under experimental conditions, dormancy can be stimulated independently of low oxygen, in media containing chelated iron, it is not excluded, that all mechanisms which stimulate the production of haemoglobin induce oviparity; in nature the most obvious one being a low dissolved oxygen concentration.

Comparative study of different geographical strains of the brine shrimp

Although brine shrimp populations are found all over the world (Abonyi, 1915; Stella, 1933; Mathias, 1937; Barigozzi, 1974), *Artemia* cysts are only exploited at a few sites in North America (Great Salt Lake, Utah, U.S.A.; San Francisco Bay, California, U.S.A.; Chaplin Lake, Saskatchewan, Canada) and in eastern Europe (Burgas-Pomorije, Bulgaria and Crimea salt lakes, U.S.S.R.) (Helfrich, 1973; Lüdskanova, 1974; Voronov, 1973).

An important fact is, that the pet market (tropical fish rearing) and all the aquaculture plants (rearing larval fishes and crustaceans) of western Europe, America and South East Asia, are relying entirely on the supply from the U.S.A., that only approximates 20-30 tons a year.

As already mentioned above, due to the expansion of both the aquaculture and the fish hobby industry, the demand for cysts has increased markedly during the last years and actually exceeds the offer.

In spite of the repeated insistence of leading authorities (in Costlow, 1969; Anonymous, 1972; Bardach *et al.*, 1972), to carry out new explorations and exploitations of the world resources of *Artemia* cysts, to alleviate the present shortage, no action has been taken yet.

In order to demonstrate the world wide distribution of *Artemia* and to carry out a comparative study on the characteristics of the different strains, we started a few years ago, to collect *Artemia* cysts from the five continents. Presently we have already gathered cysts from more than 40 different localities.

Africa :	Algeria (two sites)	
	Kenia	— Elmenteita
	Tunesia	— Tunis
America :	Argentina	— La Pampe
		— Ituzaingo
	Brasil	— Cabo Firo
		— Salinas Perynas
	Canada	— Chaplin Lake
		— Little Manitou Lake
	Mexico	— Lake Mawe
		— Yavaros, Sonora
	Peru	— Callao
		— Chilca, Lima
	Puerto Rico	— Bogueron

	U.S.A.	— San Francisco Bay
		— Great Salt Lake
		— Mono Lake
		— Moss Landing
		— Soap Lake
		— Los Angeles-area
	Venezuela	— Gulf of Cariaco
Asia :	India	— Madras
		— Madurai
		— Sambhar Lake
		— two sites in the South (locale not specified)
	Iran	— Lake Urmia
	Iraq	— Lower Mesopotamia Plain
	Israel	— Solar Lake
	Japan	— Seto Naikai coast
		— Aio
		— Tamano
	People's Republic of China	— Tsingtao
Australia :		— Bowen, Central Queensland
		— Rockhampton
		— Shark Bay
Europe :	Bulgaria	— Burgas-Pomorije
	France	— Sète
	Italy	— Comacchio
		— San Bartholomeo
	Sardegna	— Cagliari
	Spain	— Cadiz
		— Murcia
	U.S.S.R.	— Kujalnic estuary
		— Lake Tobechikskoe (Crimea)
		— Odessa

Preliminary experiments on some of these strains already revealed important differences :

- The hatching rate : under the same conditions, 50% hatching after approximately :
 - 18 hr for the San Francisco Bay strain
 - 24 hr for the Great Salt Lake strain
 - 36 hr for the Burgas-Pomorije strain
 - 48 hr for the Tsingtao strain
- The minimal illumination period, at 2,000 lux, needed for maximal hatching :
 - 5 min for the San Francisco Bay strain
 - 10 min for the Great Salt Lake strain

- The length of freshly hatched larvae : instar I nauplii of Little Manitou Lake, Comacchio, and Great Salt Lake are significantly smaller than nauplii from San Francisco Bay.
- The growth rate of the larvae is decreasing in the order mentioned for the following strains : Great Salt Lake, San Francisco Bay, Tsingtao, and Burgas-Pomorije.
- The resistance of larvae to a toxicant (solutions of lugol) : San Francisco Bay and Burgas-Pomorije nauplii are more sensitive than Tsingtao and Great Salt Lake nauplii.
- The mode of reproduction : several strains are parthenogenetic : Sète, Odessa, Comacchio, Burgas-Pomorije, Shark Bay, etc.

All the findings mentioned above were obtained from experiments carried out at 25-28 °C and 35 ‰ salinity. From the literature data and from our own experience, we known that the latter conditions are optimal for the *Artemia* strain from San Francisco Bay.

A detailed comparative study of the different races can, however, only be started after the optimal temperature-salinity ranges have been determined for each strain. From the literature on the ecology of Crustacea it appears, that the effects of temperature and salinity on factors such as e.g. growth and survival, should not be tested out separately (cf. review of Kinne, 1970).

Following the theoretical considerations on multifactorial analyses of Davies (1956), Snedecor and Cochran (1967), and Alderdice (1972), we worked out a rather simple method for the regression analysis of 2-factors experiments (Sorgeloos and Benijts-Claus, 1975). The regression equation is of the following type :

$$y = a + bx_1 + cx_2 + dx_1^2 + ex_2^2 + fx_1x_2 + g x_1^2x_2 + h x_1x_2^2 + i x_1^2x_2^2$$

in which a, b, c, ...i are constants, y is the survival (%), x_1 is the temperature (°C), and x_2 is the salinity (‰).

We report hereunder on the first results obtained with four different strains : San Francisco Bay, Great Salt Lake, Burgas-Pomorije and Tsingtao.

Materials and methods

Series of 10 freshly hatched nauplii were added to glass petri dishes of 5 cm diameter, containing 10 ml artificial seawater, formula of Dietrich and Kalle (1963). The experiments were carried out in four parallels at 16 different temperature-salinity combinations : 25, 28, 31, and 34 °C and 10, 30, 50, and 70 ‰ respectively. The lower salinities were prepared by dilution of the artificial seawater with distilled water, the higher salinities by evaporation of the artificial seawater.

The nauplii were fed twice daily with 0.1 ml of a *Scenedesmus* suspension (3 g/l). The survival rate was checked every 24 hr for 4 days, and the dead larvae siphoned off. A variance- and regression-analysis were carried out with the data obtained at the end of the 96 hr experiment, and the isopleths for the procentual survival drawn with the aid of a Wang desk-computer.

Results and discussion

1. San Francisco Bay, California, U.S.A. (Fig. 2)

In the temperature range 25–30 °C and at all salinities tested out, the survival was better than 90 %. The dominant vertical configuration of the isopleths indicates that the survival rate is mainly temperature dependent. This is confirmed by the variance-analysis that shows a significant ($P < 0.01$) linear effect of temperature: *e.g.* at 30 °C, the survival remains constant in the salinity range 20–60 ‰.

Within the temperature-salinity range tested out, the San Francisco Bay strain appears to be euryhaline and eurytherm. This finding corroborates the observations of numerous authors (*cf.* review articles by Nimura, 1967, and Ivleva, 1969).

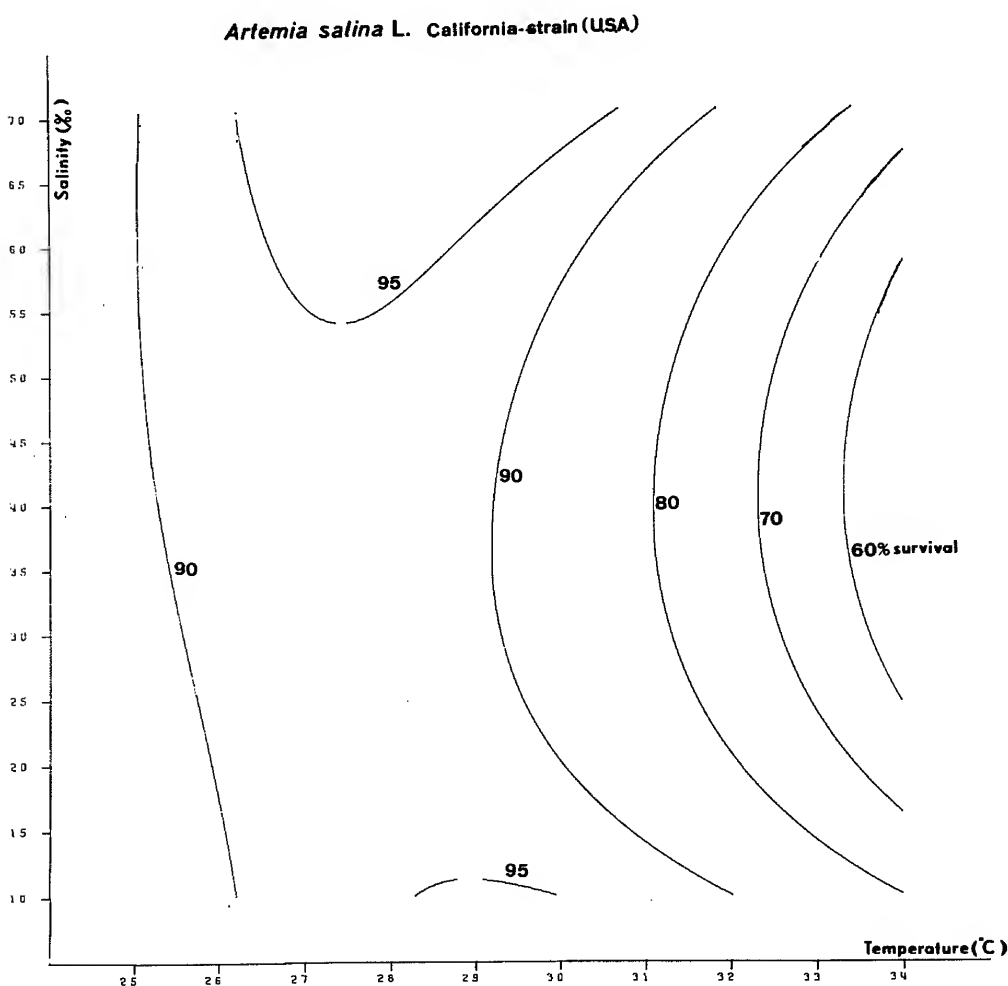


FIG. 2. Isopleths of the procentual survival of *Artemia* nauplii from San Francisco Bay, California (U.S.A.) in function of temperature and salinity.

2. Great Salt Lake, Utah, U.S.A. (Fig. 3)

The variance-analysis reveals a significant ($P < 0.01$) antagonistic interaction-effect of the linear components of both abiotic parameters : *e.g.* the negative effect of increasing temperatures on survival is counteracted by increasing salinities. This is further confirmed by the typical "rising-ridge" configuration of the isopleths (*cf.* Davies, 1956). From Fig. 3 it is clear that this Utah strain definitely prefers higher salinities. Analogous results with the Great Salt Lake strain have been obtained by Von Hentig (1971) who reported that growth and reproduction are minimal below 30‰, and who found the highest production of offspring at 70‰ salinity.

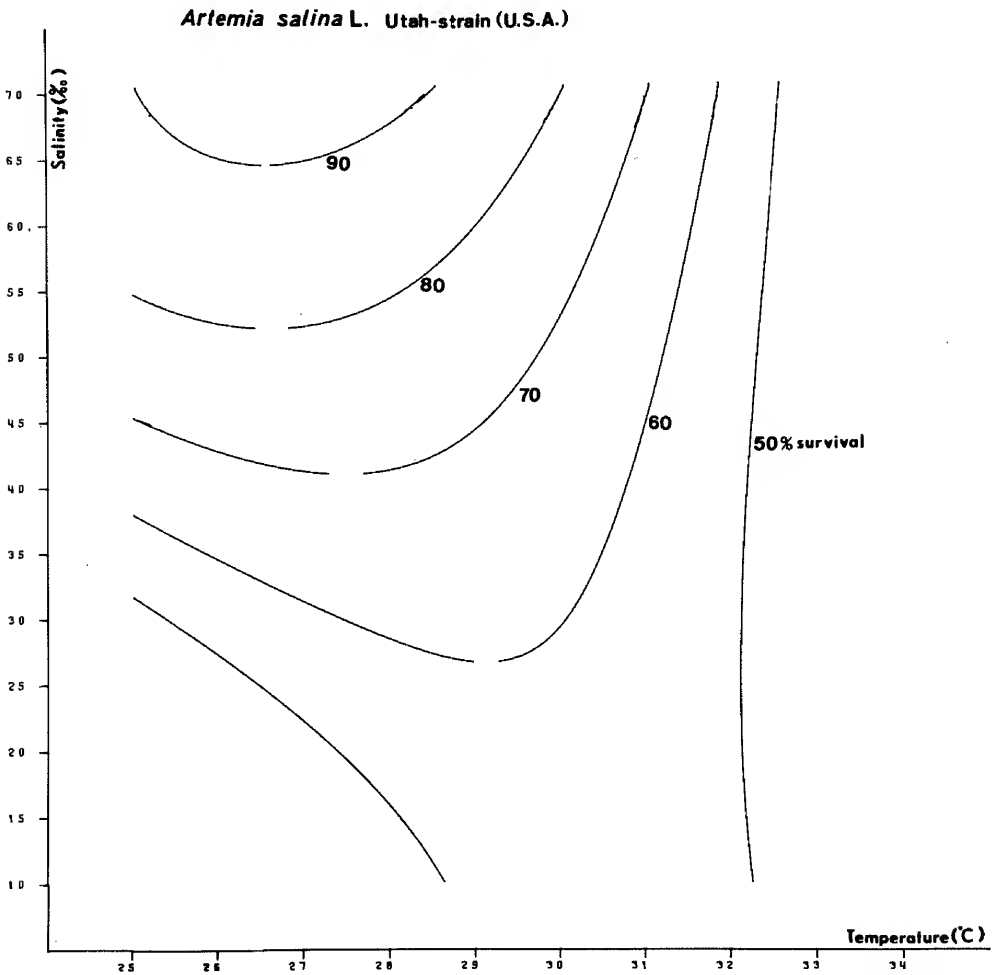


FIG. 3. Isopleths of the procentual survival of *Artemia* nauplii from Great Salt Lake, Utah (U.S.A.) in function of temperature and salinity.

3. Burgas-Pomorije, Bulgaria (Fig. 4)

Survival is minimal below 20‰ salinity and at temperatures exceeding 31 °C. The survival rate is significantly influenced by the linear components of both abiotic parameters. The configuration of the isopleths reveals the existence of an optimal temperature-salinity combination: at 26-27 °C and 50-55‰ salinity, the survival is higher than 85 %. When shifting from that particular combination to other temperatures and salinities, the occurring increase in mortality indicates, that the Burgas-Pomorije strain is stenohaline and stenotherm. The only information available on this strain comes from Lüdskanova and Joshev (1972) who reported optimal hatching conditions at 22 °C and 47 ‰.

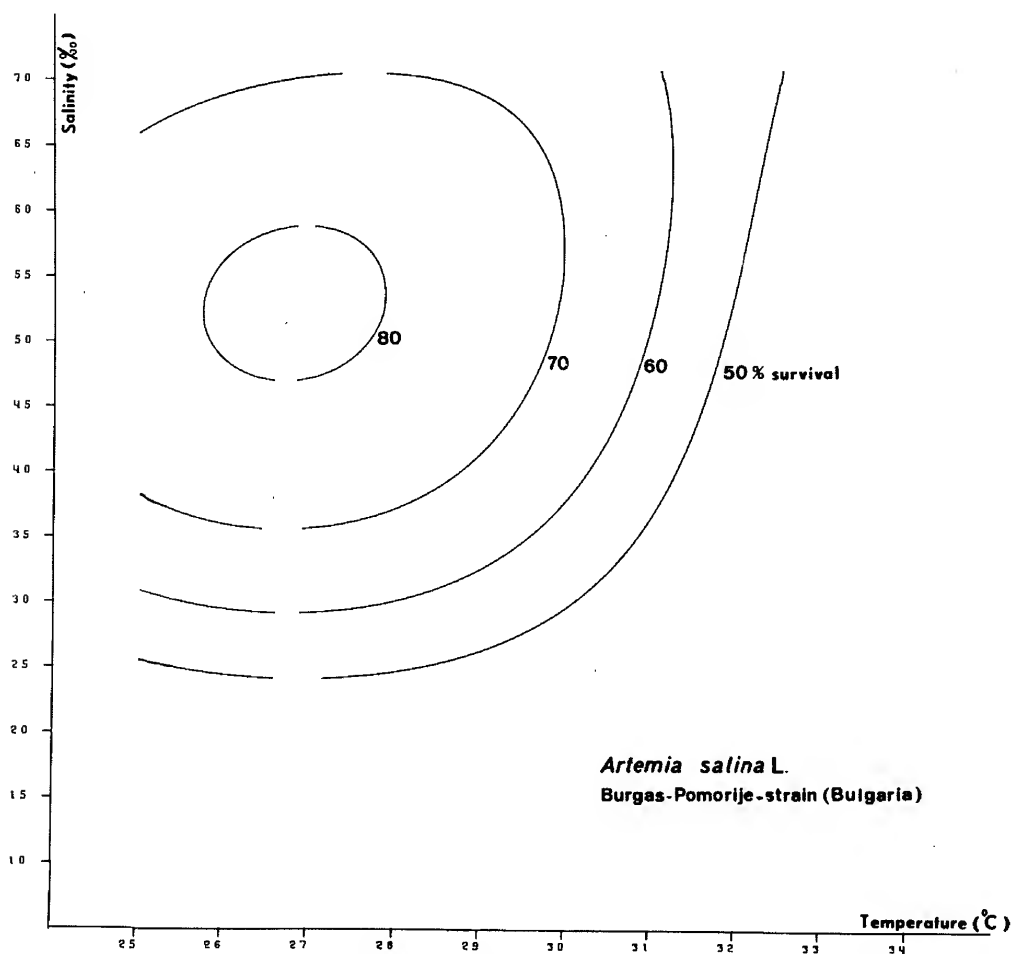


FIG. 4. Isopleths of the procentual survival of *Artemia* nauplii from Burgas-Pomorije (Bulgaria) in function of temperature and salinity.

4. Tsingtao, People's Republic of China (Fig. 5)

The survival rate of the nauplii is minimal at salinities below 40‰. The linear component of the factor salinity indicates, that this parameter is dominant as far as the survival rate is concerned. From the configuration of the isopleths in Fig. 5 it appears that the mortality decreases with increasing salinity and decreasing temperature. At the highest salinities tested out, this particular strain can be considered as being eurytherm.

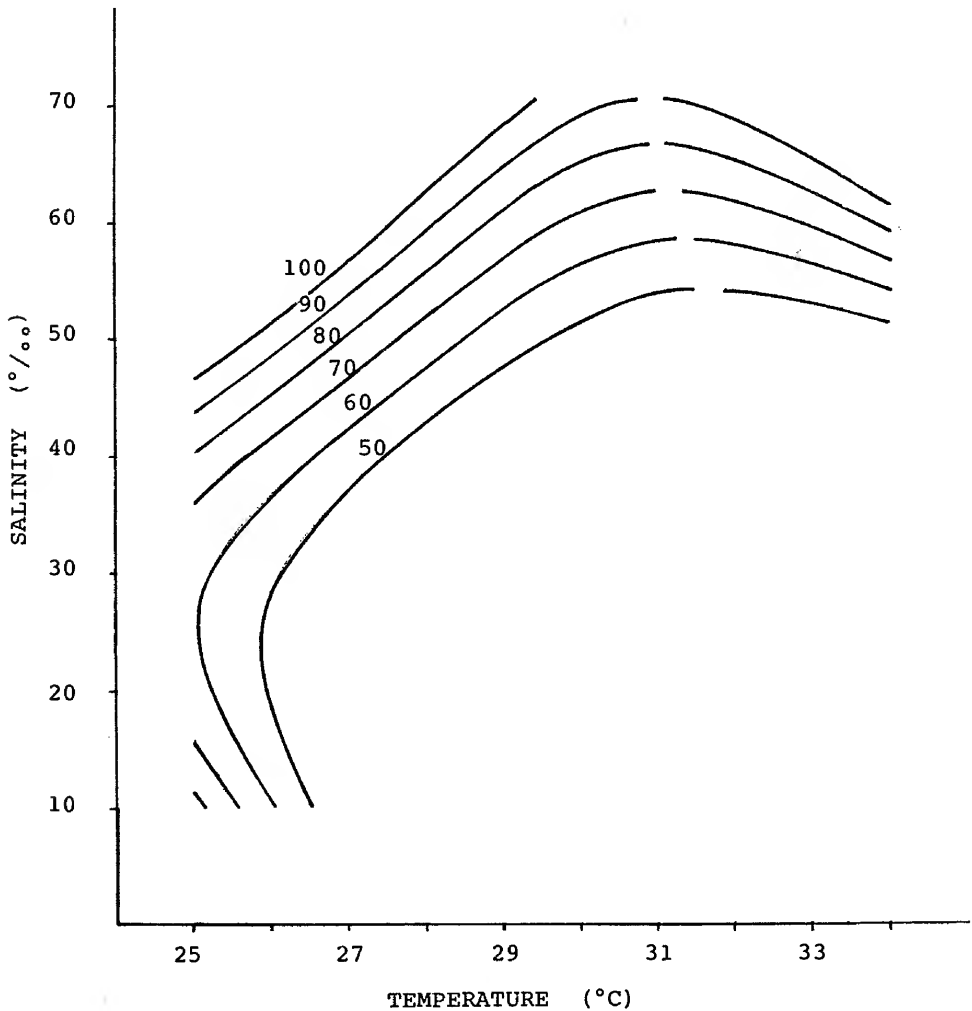


FIG. 5. Isopleths of the procentual survival of *Artemia* nauplii from Tsingtao (People's Republic of China) in function of temperature and salinity.

Conclusions

From the foregoing it is clear that the temperature- and salinity tolerance of the first larval stages of *Artemia salina* can differ considerably from one strain to another.

Although in the literature, the brine shrimp is considered as a typical euryhaline and eurytherm organism (cf. review by Bayly, 1972; salinity-tolerance range of 3-300‰), it appears from the preliminary data given in this paper, that this certainly is not a general rule. Indeed, the strain on which most ecological research has been performed, namely the one from San Francisco Bay, is euryhaline; other strains do not thrive in relatively low salinities (Great Salt Lake and Tsingtao) and the strain from Burgas-Pomorije is even stenotherm and stenohaline.

Recently a detailed comparative study of the 40 available strains has been started in our laboratory. These studies, aimed at determining the ecological preferences, the growth and reproductive potential, and the nutritional value of each strain, shall hopefully lead to the selection of the *Artemia* strains best suited for aquacultural purposes.

Besides the evaluation of new harvesting areas, which shall be the next step, this study can also lead to transplantation experiments with inoculation of particular strains into new biotopes or in places where, for example, the autochthonous *Artemia* strains are not very well suited for aquaculture due to e.g. low nutritional value or limited resistance.

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